

model explains 16.5% of the temperature variance in the first sub-period and 18.4% in the verification period.

In general, only the model 1 (i.e.  $t$  alone) showed significant results. Though variance explained by the other two models was high in some cases, significance level of these lagged variables in both models failed to equal the pre-set limit, i.e.  $t$ -value significant at  $P < 0.05$ .

For the reconstructed May temperature (Figure 5), calibration performed well for the verification period 1891–1920 that was not used in calibration (correlation coefficient 0.439; reduction of error 0.166; and product mean  $t = 1.841$ , Table 3).

The present study suggests that *LAGR* has good prospect in understanding fine resolution climatic changes from the Eastern Himalayan region using tree ring width data. It has all the characteristics which make it a potential parameter for the reconstruction of May temperature. Moreover distinct demarcation of early wood and late wood cells in the rings suggests that early wood and late wood measurements separately could be additional parameters, besides total ring width. Future analysis using these two tree ring parameters may help us understand climatic changes of other months also. Although the tree ring data discussed here are not enough (1896–1995 AD) to build long climatic records, these data could be extended further through cross dating with additional collections from older stands of this tree from the region. Thus the present tree ring study signifies its future perspective of long summer temperature reconstruction of this region.

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## **Organic manuring in rice-based cropping system: Effects on soil microbial biomass and selected enzyme activities**

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**Soils exclusively amended with poultry manure, farmyard manure, sesbania and gliricidia for three successive rice–rice–cowpea cropping systems were incubated as such or after fresh addition of the respective organic manure at  $37 \pm 1^\circ\text{C}$  under submergence. The treatments also included fresh incorporation of these organic manures into soils with no amendment history. Soil microbial biomass (total, fungi, actinomycetes, bacteria), biomass C, N flush and the activities of enzymes like amylase, cellulase, arylsulfatase,  $\beta$ -glucosidase and inorganic pyrophosphatase were determined at different stages of incubation and the data pertaining to peak enzyme activity (30th day) are reported.**

**Soils amended with organic manures consistently registered significantly greater microbial biomass, biomass C, N flush and enzyme activities compared to the unamended soil. All the enzymes were significantly activated to different degrees, which however, varied with the type of organic manure added to soils. Positive relationships between relevant soil properties and enzyme activities suggest that addition of organic manures increased microbial activity/diversity and C turnover, which subsequently led to greater enzyme synthesis and accumulation in the soil matrix. It is, therefore, apparent that soil microbial biomass and enzyme activities are sensitive even to short-term organic manuring.**

IT is well known that intensive cultivation has led to a rapid decline in organic matter and nutrient levels be-

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\*For correspondence.

**Table 1.** Some soil properties and enzyme activities at zero-time of incubation

| Soil | pH   | Mineral N<br>( $\mu\text{g/g}$ ) | Organic C<br>( $\mu\text{g/g}$ ) | Total N<br>( $\mu\text{g/g}$ ) | $\beta$ -glucosidase <sup>a</sup> | Arylsulphatase <sup>b</sup> | Cellulase <sup>c</sup> | Amylase <sup>d</sup> | Inorganic<br>pyrophosphatase <sup>e</sup> |
|------|------|----------------------------------|----------------------------------|--------------------------------|-----------------------------------|-----------------------------|------------------------|----------------------|---|
| NAC  | 5.72 | 10.13                            | 6213                             | 514                            | 72.3                              | 61.2                        | 0.51                   | 0.44                 | 96.9                                      |
| APM  | 6.12 | 15.75                            | 7014                             | 712                            | 90.5                              | 83.1                        | 0.68                   | 0.46                 | 192.8                                     |
| AFYM | 6.10 | 15.80                            | 8302                             | 714                            | 98.7                              | 94.5                        | 0.70                   | 0.49                 | 216.6                                     |
| AGL  | 6.32 | 17.52                            | 8600                             | 739                            | 126.5                             | 120.9                       | 0.85                   | 0.56                 | 231.8                                     |
| ASES | 6.37 | 18.14                            | 8611                             | 752                            | 129.3                             | 132.0                       | 0.87                   | 0.58                 | 240.3                                     |

<sup>a,b</sup> $\mu\text{g } p\text{-nitro phenol g}^{-1} \text{ soil h}^{-1}$ ; <sup>c,d</sup> $\text{mg glucose g}^{-1} \text{ soil } 24 \text{ h}^{-1}$ ; <sup>e</sup> $\mu\text{g ortho-P g}^{-1} \text{ soil } 5 \text{ h}^{-1}$ .

NAC, Non amended control; APM, Soil previously amended with poultry manure; AFYM, Soil previously amended with farm yard manure; AGL, Soil previously amended with gliricidia; ASES, Soil previously amended with sesbania.

**Table 2.** Treatment details

*Soil with no amendment history<sup>a</sup> + fresh incorporation<sup>c</sup>*

NAC  
NAC + PM (poultry manure)  
NAC + FYM (farmyard manure)  
NAC + GL (gliricidia)  
NAC + SES (sesbania)

*Soil with previously amended history<sup>b</sup> + fresh incorporation<sup>c</sup>*

APM  
APM + PM  
AFYM  
AFYM + FYM  
AGL  
AGL + GL  
ASES  
ASES + SES

<sup>a</sup>Soils collected from sites where no organic manure/chemical fertilizers were applied.

<sup>b</sup>Soils collected at the end of five successive rice–rice–cowpea cropping systems; these soils were amended with the respective organic manures @  $3.1 \text{ t ha}^{-1}$  poultry manure,  $15 \text{ t ha}^{-1}$  farmyard manure,  $11 \text{ t ha}^{-1}$  each of sesbania and gliricidia, 10 days before transplanting of each rice crop.

<sup>c</sup>Fresh incorporation of the respective organic manure at rates given above, at zero-time of incubation.

sides affecting soil physical properties. Conversely, crop residue management practices influence agricultural sustainability by improving physical, chemical and biological properties of soils<sup>1</sup>. However, a better understanding of nutrient cycling and the factors governing their decomposition in soil is imperative for implementing sustainable management practices.

Nutrient cycling in soil involves chemical, biochemical and physico-chemical reactions, with the biochemical reactions being catalysed by soil enzymes primarily associated with viable cells of microbial origin<sup>2</sup>. Therefore, any factor that affects soil microbial population will necessarily alter soil enzyme activity.

Though several studies have documented the effects of crop residue/organic manure additions on soil microbial and enzyme activities<sup>3–5</sup>, systematic studies are needed across ecosystems and short-term/long-term soil

management sites before these data can be used as reliable indicators of soil quality<sup>6</sup>. Besides, information on soil microbial and enzyme activities in different agroecosystems, especially the widely prevalent rice-based cropping systems of Asia is limited.

Therefore, the present study was conducted with the primary objective of determining the effect of successive additions of organic manures on microbial biomass and activities of some enzymes in soils of a rice–rice–cowpea cropping system. The other objective was to examine the relationship between enzyme activities and relevant soil properties like pH, organic C and total N content.

An organic manuring study was initiated in 1993 at the Central Agricultural Research Institute (CARI), Port Blair, India consisting of incorporation of poultry manure containing 3.2% N @  $3.1 \text{ t ha}^{-1}$ , farmyard manure containing 0.7% N @  $15 \text{ t ha}^{-1}$ , *Gliricidia maculata* containing 2% N @  $11 \text{ t ha}^{-1}$  and *Sesbania rostrata* containing 2% N @  $11 \text{ t ha}^{-1}$ , applied alone and in combination with varying levels of urea into plots of a rice–rice–cowpea system. In a year, two rice crops are grown in succession (May–August and August–January) followed by a residual cowpea crop (January–April). The organic manures are incorporated into the soil 10 days before transplantation of each rice crop. After five successive years of rice–rice–cowpea cropping system, soil samples (0–30 cm) were collected from plots exclusively applied with the respective organic manures and stored at  $4^\circ\text{C}$  for not more than one week before initiation of the incubation study. Important properties of the initial soil samples are given in Table 1.

For the incubation study, the soils (1000 g each, air-dried;  $< 2 \text{ mm}$ ) were either incubated as such or treated with the respective organic manure (oven-dried at  $70^\circ\text{C}$ ;  $< 2 \text{ mm}$ ) at rates equivalent to those applied for each rice crop and mixed thoroughly. The treatment also included fresh incorporation of the organic manures into soils with no amendment history. The treatment details are given in Table 2. Three replicate samples of each treatment were placed in polythene containers, incubated at  $37 \pm 1^\circ\text{C}$  under submergence and assayed for

**Table 3.** Relevant properties and microbial characteristics of soils at 30th day of incubation

| Treatment <sup>a</sup> | pH   | Mineral N<br>( $\mu\text{g g}^{-1}$ ) | Organic C<br>( $\mu\text{g g}^{-1}$ ) | Total N<br>( $\mu\text{g g}^{-1}$ ) | Biomass C<br>( $\mu\text{g g}^{-1}$ ) | N flush<br>( $\mu\text{g g}^{-1}$ ) | Plate count <sup>b</sup> |              |              |              |
|------------------------|------|---------------------------------------|---------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|--------------------------|--------------|--------------|--------------|
|                        |      |                                       |                                       |                                     |                                       |                                     | T ( $10^7$ )             | F ( $10^5$ ) | A ( $10^6$ ) | B ( $10^6$ ) |
| NAC                    | 5.60 | 9.32                                  | 6102                                  | 504                                 | 183                                   | 2.2                                 | 1.24                     | 1.86         | 2.61         | 0.9          |
| NAC + PM               | 5.52 | 15.73                                 | 6921                                  | 813                                 | 204                                   | 11.6                                | 2.98                     | 3.70         | 4.61         | 2.6          |
| NAC + FYM              | 5.51 | 15.82                                 | 8102                                  | 820                                 | 251                                   | 10.8                                | 3.12                     | 3.81         | 4.61         | 2.6          |
| NAC + GL               | 5.32 | 17.32                                 | 8600                                  | 840                                 | 256                                   | 14.0                                | 3.91                     | 4.71         | 4.82         | 4.0          |
| NAC + SES              | 5.32 | 18.56                                 | 8611                                  | 845                                 | 257                                   | 15.3                                | 3.94                     | 4.86         | 4.83         | 3.6          |
| APM                    | 6.04 | 14.03                                 | 7692                                  | 750                                 | 224                                   | 4.6                                 | 2.21                     | 3.20         | 3.63         | 1.5          |
| APM + PM               | 5.82 | 18.12                                 | 8392                                  | 841                                 | 252                                   | 13.7                                | 4.23                     | 5.02         | 5.12         | 3.7          |
| AFYM                   | 6.00 | 12.00                                 | 7504                                  | 753                                 | 229                                   | 4.4                                 | 2.21                     | 3.20         | 3.63         | 1.5          |
| AFYM + FYM             | 5.82 | 17.13                                 | 9702                                  | 962                                 | 291                                   | 12.1                                | 4.25                     | 5.10         | 5.42         | 4.6          |
| AGL                    | 6.14 | 15.21                                 | 8013                                  | 799                                 | 239                                   | 5.3                                 | 2.15                     | 3.31         | 4.11         | 2.7          |
| AGL + GL               | 5.72 | 19.21                                 | 10490                                 | 1033                                | 312                                   | 16.0                                | 5.23                     | 6.23         | 6.60         | 4.3          |
| ASES                   | 6.21 | 16.32                                 | 8112                                  | 809                                 | 243                                   | 5.4                                 | 2.17                     | 3.52         | 4.15         | 2.7          |
| ASES + SES             | 5.84 | 21.32                                 | 10602                                 | 1052                                | 318                                   | 17.0                                | 6.02                     | 6.53         | 6.90         | 4.6          |
| LSD 0.05               | 0.20 | 1.20                                  | 303                                   | 42                                  | 10                                    | 0.5                                 | 0.10                     | 0.11         | 0.12         | 0.3          |

<sup>a</sup>For description of treatments refer to Table 2.

<sup>b</sup>T, Total; F, Fungi; A, Actinomycetes; B, Bacteria.

**Table 4.** Enzyme activity in soils at 30th day of incubation

| Treatment  | $\beta$ -glucosidase <sup>a</sup> | Arylsulphatase <sup>b</sup> | Cellulase <sup>c</sup> | Amylase <sup>d</sup> | Inorganic pyrophosphatase <sup>e</sup> |
|------------|-----------------------------------|-----------------------------|------------------------|----------------------|--|
| NAC        | 62.5 (100)                        | 51.4 (100)                  | 0.45 (100)             | 0.40 (100)           | 83.6 (100)                             |
| NAC + PM   | 141.8 (227)                       | 140.4 (273)                 | 1.02 (249)             | 0.46 (127)           | 241.8 (290)                            |
| NAC + FYM  | 143.2 (229)                       | 143.2 (278)                 | 1.12 (249)             | 0.51 (128)           | 272.4 (327)                            |
| NAC + GL   | 211.3 (338)                       | 214.1 (416)                 | 1.36 (302)             | 0.68 (170)           | 323.8 (388)                            |
| NAC + SES  | 218.2 (349)                       | 230.7 (449)                 | 1.47 (327)             | 0.78 (195)           | 343.3 (412)                            |
| APM        | 86.1(138)                         | 73.7 (143)                  | 0.60 (148)             | 0.46 (115)           | 183.5 (220)                            |
| APM + PM   | 155.8 (249)                       | 161.2 (313)                 | 1.28 (286)             | 0.60 (149)           | 311.4 (373)                            |
| AFYM       | 88.9 (142)                        | 82.0 (159)                  | 0.68 (152)             | 0.46 (116)           | 191.8 (230)                            |
| AFYM + FYM | 154.3 (247)                       | 155.7 (303)                 | 1.30 (289)             | 0.63 (157)           | 283.5 (340)                            |
| AGL        | 108.4 (173)                       | 115.4 (224)                 | 0.83 (184)             | 0.48 (121)           | 221.0 (265)                            |
| AGL + GL   | 226.7 (362)                       | 240.5 (467)                 | 1.73 (384)             | 0.78 (195)           | 411.4 (493)                            |
| ASES       | 109.8 (175)                       | 118.2 (230)                 | 0.85 (189)             | 0.49 (122)           | 230.7 (277)                            |
| ASES + SES | 240.6 (384)                       | 272.4 (530)                 | 1.96 (436)             | 0.82 (206)           | 443.4 (532)                            |
| LSD 0.05   | 15.3                              | 19.5                        | 0.12                   | 0.03                 | 15.2                                   |

<sup>a,b</sup> $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ soil h}^{-1}$ ; <sup>c,d</sup> $\text{mg glucose g}^{-1} \text{ soil } 24 \text{ h}^{-1}$ ; <sup>e</sup> $\mu\text{g ortho-P g}^{-1} \text{ soil } 5 \text{ h}^{-1}$ .

Figures in parentheses indicate mean percentage of control.

enzyme activity at 30th, 60th, 90th and 120th day of incubation. Likewise, three other replicate samples of each treatment were used for determining microbial counts, N flush and biomass C.

Except for time-zero sampling, soil analyses were performed on moist samples throughout. Mineral N,  $\text{NH}_4 + \text{NO}_3\text{-N}$ , total N and organic carbon were determined using standard methods<sup>7</sup>. Soil pH was measured with a glass electrode using 1:2.5 soil:water ratio.

All biological estimations were conducted on moist samples. The serial dilution technique was used for all soil plate counts. Soil micro-organisms, viz. total aerobes, bacteria, fungi and actinomycetes were determined using standard methods<sup>7</sup>. Microbial biomass was

estimated by using a modified method of Bolton *et al.*<sup>8</sup>. The method of Ross *et al.*<sup>9</sup> was used to determine the N flush.

Selected soil enzymes were assayed on moist soil samples throughout. Cellulase and amylase were assayed as described by Pancholy and Rice<sup>10</sup>. Arylsulphatase, inorganic pyrophosphatase and  $\beta$ -glucosidase were assayed by methods previously described<sup>11</sup>. All values reported are on a dry weight  $\text{g}^{-1}$ , oven ( $110^\circ\text{C}$ ) dry weight of soil basis.

The data were analysed with the ANOVA procedure<sup>12</sup> using an *F*-test to determine if there were any significant differences (0.05 level). Correlations among the parameters were determined on mean values of three replications.

Though the enzymes were assayed at the 30th, 60th, 90th and 120th day of incubation, we report here only the data pertaining to the 30th day, because peak activity was observed at this stage and also enzyme activities showed identical trends at all stages of incubation. Mineral N, total N, organic C and biomass C were greatest at the 60th day, remained almost consistent at the 90th day, followed by a gradual decline at the 120th day of incubation (data not given). However, in order to draw a plausible relationship between peak enzyme activities and the soil properties, we report only the data pertaining to the 30th day of incubation.

The data on fungi, actinomycetes, bacteria and total count (Table 3) indicate that incorporation of organic manures provided a conducive environment for microbial proliferation due to increased organic C, mineral N and total N content of soils. The biomass C and N flush values, which are a measure of microbial population, were also greater in amended soils. The biomass values recorded in this study, on an average constituted 3.0% of the soil organic C. In general, biomass C constitutes 2–4% of the soil organic carbon<sup>13</sup>.

Enzyme activities were also consistently greater in the amended soils. The influence was more evident when the activity values were expressed as per cent variation from activity values of control (Table 4). Greater enzyme activity in the amended soils was the result not only of a large microbial biomass, but also of higher amounts of endoenzymes and greater enzyme production by this microbial biomass. Besides, increased levels of accumulated enzymes in the soil matrix<sup>14</sup> and more importantly, direct contribution of enzymes by the organic manures themselves, might also be responsible for greater soil enzyme activity<sup>3</sup>. Since enzyme activities are highly correlated with both microbial respiration and total biomass<sup>15</sup>, it is obvious that greater biological activity has been established in the amended soils. Variation in enzyme activities among the amended soils might possibly be due to variations in the organic matter content, as well as the type of organic manure added to the soils<sup>16</sup>. It, therefore, appears that the type of organic manure added during succession determines the activity gradient of the enzymes under study.

**Table 5.** Correlation coefficient ( $r$ )<sup>a</sup> between enzyme activities and relevant soil properties

|                           | pH                  | Organic C          | Total N            | Mineral N          |
|---------------------------|---------------------|--------------------|--------------------|--------------------|
| $\beta$ -glucosidase      | -0.488 <sup>c</sup> | 0.805 <sup>b</sup> | 0.807 <sup>b</sup> | 0.896 <sup>b</sup> |
| Aryl sulphatase           | -0.421 <sup>c</sup> | 0.825 <sup>b</sup> | 0.825 <sup>b</sup> | 0.916 <sup>b</sup> |
| Cellulase                 | -0.357 <sup>c</sup> | 0.883 <sup>b</sup> | 0.883 <sup>b</sup> | 0.929 <sup>b</sup> |
| Amylase                   | -0.391 <sup>c</sup> | 0.869 <sup>b</sup> | 0.796 <sup>b</sup> | 0.863 <sup>b</sup> |
| Inorganic pyrophosphatase | -0.255 <sup>c</sup> | 0.897 <sup>b</sup> | 0.917 <sup>b</sup> | 0.957 <sup>b</sup> |

<sup>a</sup> $n = 13$ ; <sup>b</sup>Significant at  $P < 0.01$ ; <sup>c</sup> Not significant.

The enzyme activities did not show any significant relationship with pH (Table 5). Earlier studies have also shown no relationship between soil pH and cellulase and amylase activities<sup>10</sup> or  $\beta$ -glucosidase activity<sup>16</sup>. Significant and positive correlations between the other properties and enzyme activities (Table 5) indicate that incorporation of organic manures had contributed significantly to soil organic C, total N and mineral N, which in turn led to greater microbial proliferation and subsequently to greater enzyme synthesis and accumulation. Higher organic C levels stimulate microbial activity and provide a favourable environment for enzyme synthesis and accumulation in the soil matrix, since organic constituents are thought to be important in forming stable complexes with free enzymes<sup>17</sup>. Similarly, positive relationship between total N content and enzyme activity indicates higher C turnover in soils amended with organic manures compared to control.

It is, therefore, apparent that addition of organic manure to soils enhances soil organic C status and microbial activity/diversity, which subsequently enhance soil enzyme synthesis and accumulation. This in turn would bolster the soil's capability to cycle and provide nutrients for crop growth. Though the present results cannot be taken as a consistent index for evaluation of soil quality it can, however, be inferred that soil microbial biomass and enzyme activity are sensitive to even short-term organic manuring. Future research must concentrate on deriving strong correlation between soil enzyme activity–fertility–crop productivity, taking into consideration all the factors that might confound or override the relationship between soil biological activity and crop productivity.

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